NVI 5183.1
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Charles S. Schasteen, et al. Art Unit 1645
Serial No. 10/005,510
Filed November 8, 2001
Confirmation No. 9657
For METHODS AND COMPOSITIONS FOR THE CONTROL OF COCCIDIOSIS
Examiner Vanessa L. Ford

December 13, 2006

APPEAL BRIEF

John K. Roedel, Jr., Reg. No. 25,914
SENNIGER POWERS
One Metropolitan Square, 16th Floor
St. Louis, Missouri 63102
(314) 231-5400

TABLE OF CONTENTS

TABLE OF AUTHORITIES

REFERENCES

CASES

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Charles S. Schasteen, et al. Art Unit 1645
Serial No. 10/005,510
Filed November 8, 2001
Confirmation No. 9657
For METHODS AND COMPOSITIONS FOR THE CONTROL OF COCCIDIOSIS
Examiner Vanessa L. Ford

APPEAL BRIEF

This is an appeal from the final rejection of the claims of the above-identified application made in the Office action dated June 13, 2006. A Notice of Appeal was filed on September 13, 2006.

I. REAL PARTY IN INTEREST

The real party in interest in connection with the present appeal is Novus International, Inc. of 530 Maryville Centre Drive, St. Louis, Missouri 63141, a corporation of the state of Delaware, owner of a 100 percent interest in the pending application.

II. RELATED APPEALS AND INTERFERENCES

Applicants are unaware of any pending appeals or interferences which may be related to, directly affect or be directly affected by, or have a bearing on, the Board's decision in the pending appeal.

III. STATUS OF CLAIMS

Claims 1, 4-30, 113-116, 118-119, 136-146, 148-150, and 153-154 are currently pending in the application, claims 2-3, 31-112, 117, 120-135, 147, and 151-152 have been cancelled, and claims 144-145 have been withdrawn. A copy of the pending claims appears in the Claims Appendix of this Brief.

Claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 stand rejected under 35 U.S.C. §102(a) as being anticipated by Conkle, et al. (WO 00/50072), and under 35 U.S.C. §102(b) as being anticipated by Evans, et al. (WO 96/40234).

Claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 stand rejected under 35 U.S.C. §103(a): (1) over Conkle, et al. (WO 0050072) in view of Brown, et al. (U.S. Patent No. 6,019,985), and (2) over Evans, et al. (WO 96/40234) in view of Brown, et al. (U.S. Patent No. 6,019,985).

The rejections of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 under 35 U.S.C. §102(a) and §102(b) and the rejection of claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 under 35 U.S.C. §103(a) are being appealed.

IV. STATUS OF AMENDMENTS

No amendments have been filed after the final rejection.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The following summary correlates claim elements to specific embodiments described in the application specification, but does not in any manner limit claim interpretation. Rather, the

following summary is provided only to facilitate the Board's understanding of the subject matter of this appeal.

Claim 1 of the application is directed to a composition for the prevention or control of coccidiosis comprising viable sporulated oocysts (*see, e.g.,* Specification, p. 6, line 14) that are derived from an oocysts source comprising bacterial contamination (*see, e.g.,* Specification, p. 13, lines 6-8) and comprise at least one species of protozoa known to cause coccidiosis (*see, e.g.,* Specification at p. 6, lines 14-15), wherein said composition is sterile and contains at least about 10,000 oocysts per milliliter and less than about 0.4% by weight of alkali metal dichromate (*see* Specification, p. 46, lines 23 and 26-27), said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores (*see, e.g.,* Specification, p. 34, line 25 to p. 35, line 19; *see also* Specification, p. 38, line 22 to p. 39, line 13).

Claim 9 of the application is directed to a composition for the prevention or control of coccidiosis comprising viable sporulated oocysts (*see, e.g.,* Specification, p. 6, line 14) that are derived from an oocyst source comprising bacterial contamination (*see, e.g.,* Specification, p. 13, lines 6-8) and comprise at least one species of protozoa known to cause coccidiosis (*see, e.g.,* Specification at p. 6, lines 14-15), wherein said composition is sterile and contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate (*see* Specification, p. 57, claim 9),

said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores (*see, e.g.,* Specification, p. 34, line 25 to p. 35, line 19; *see also* Specification, p. 38, line 22 to p. 39, line 13).

Claim 10 of the application is directed to a composition for the prevention or control of coccidiosis comprising viable sporulated oocysts (*see, e.g.,* Specification, p. 6, line 14) that are derived from an oocysts source comprising bacterial contamination (*see, e.g.,* Specification, p. 13, lines 6-8) and comprise at least one species of protozoa known to cause coccidiosis (*see, e.g.,* Specification at p. 6, lines 14-15), wherein said composition is sterile and contains less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst (*see* Specification, p. 47, lines 19-20 and p. 57, claim 10), said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores (*see, e.g.,* Specification, p. 34, line 25 to p. 35, line 19; *see also* Specification, p. 38, line 22 to p. 39, line 13).

Claim 23 of the present application is directed to the composition as set forth in claim 14, further comprising a composition that ameliorates a decline in post challenge

performance (*see* Specification, p. 44, lines 3-22 and p. 58, claim 23).

Claim 30 of the present application is directed to a composition as set forth in claim 29, further comprising a composition that ameliorates a decline in post challenge performance (*see* Specification, p. 44, lines 3-22 and p. 60, claim 30).

Claim 113 of the present application is directed to a kit for the prevention or control of coccidiosis comprising, the composition of claim 1; and instructions for administration of said composition to an animal (*see, e.g.,* Specification, p. 7, lines 28 and 30-31, and p. 72, claim 113).

Claim 139 of the present application is directed to a composition as set forth in claim 137 wherein said composition comprises viable sporulated oocysts of *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella* in a ratio defined by the minimum immunizing dose and amount determined by storage half life determinations (*see* Specification, p. 45, line 17-27, and p. 75, claim 139).

Claim 142 of the present application is directed to a composition as set forth in claim 137 comprising a composition which ameliorates a decrease in post challenge performance (*see* Specification, p. 44, lines 3-22 and p. 76, claim 142).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The issue on appeal is whether the subject matter of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 satisfy the requirements of 35 U.S.C. §102(a) and §102(b), and whether the subject matter of claims 1, 4-30, 113-

116, 118-119, 136-143, 146, 148-150, and 153-154 satisfy the requirements of 35 U.S.C. §103(a). Accordingly:

A. Applicants appeal the rejection of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 as anticipated by Conkle, et al. (WO 00/50072) under 35 U.S.C. §102(a).

B. Applicants appeal the rejection of claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 as unpatentable under 35 U.S.C. §103(a) over Conkle, et al. (WO 00/50072) in view of Brown, et al. (U.S. Patent No. 6,019,985).

C. Applicants appeal the rejection of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 as anticipated by Evans, et al. (WO 96/40234) under 35 U.S.C. §102(b).

D. Applicants appeal the rejection of claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 as unpatentable under 35 U.S.C. §103(a) over Evans, et al. (WO 96/40234) in view of Brown, et al. (U.S. Patent No. 6,019,985).

VII. ARGUMENT

A. Claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 are patentable under 35 U.S.C. §102(a) over Conkle, et al. (WO 00/50072)

Independent claim 1 is directed to a composition for the prevention or control of coccidiosis comprising viable sporulated oocysts that are derived from an oocysts source comprising bacterial contamination and comprise at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains at least about 10,000 oocysts per milliliter and less than about 0.4% by weight of

alkali metal dichromate, said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores.

As defined in claim 1, the claimed composition is not only substantially free of live bacteria that can be killed by sodium dichromate, but is also substantially free of dead bacteria and cellular debris that are derived from the source and remain in a vaccine composition after chemical treatment.

Conkle, et al. describe a method for preparing a vaccine against avian coccidiosis. The method of Conkle, et al. includes obtaining coccidial oocysts from a fecal suspension, homogenizing the fecal suspension, separating the oocysts from the fecal debris, sporulating the oocysts, bleaching the sporulated oocysts, washing the bleached oocysts, and concentrating the sterile washed oocysts.

Significantly, however, Conkle, et al. fail to disclose or suggest an oocyst-containing composition that is substantially free of bacterial contaminants that are:

> "present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane with a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores"

Conkle, et al. state that oocysts may be washed following sporulation to reduce the residual oxidant concentration to an acceptable level. Serial washings may be conducted, preferably by membrane filtration, and more preferably by diafiltration.

Serial washing or diafiltration may also be used after bleaching to reduce the residual oxidant concentration in the bleached suspension (e.g., the concentration of sodium hypochlorite in the suspension), to an acceptable level.[1]

It is important to understand that the washing and filtration steps of Conkle, et al. do not render the Conkle, et al. vaccine "substantially free of bacterial contaminants." Significantly, Conkle, et al. fail to disclose or suggest the use of a filter pore size small enough to prevent sporulated oocysts from entering the pores, but large enough to allow bacteria to pass through the pores. Rather, the only mention in Conkle, et al. of pore size is a statement that in the case of membrane filtration, "the membrane pore size is selected to allow passage of solutes through the membrane while restricting the passage of the oocysts from one side of the membrane to the other."[2] There is no statement or suggestion in Conkle, et al. that the pore size should be large enough to allow the passage of bacteria, as well as solutes. In fact, such a pore size would not be necessary to achieve the stated purpose of washing in Conkle, et al., i.e., to reduce the residual oxidant concentration to an acceptable level.

Furthermore, it is important to understand that "bacterial contaminants," as specified in claim 1 encompass not only live bacteria, but non-viable contaminants such as dead bacteria and cellular debris that remain after treatment with an anti-bacterial agent. As specified in claim 1, the pore size of the filter membrane used during tangential flow filtration is large enough to allow bacteria to pass through. As a consequence, the

[1] "Following bleaching, the bleached suspension is washed, if necessary, to reduce the residual oxidant concentration to an acceptable level." Conkle, et al., p. 8, ln. 33-35.
[2] Conkle, et al., p. 8, ln. 19-20 (emphasis added).

oocysts retained by the filter membrane have been separated from both viable and non-viable contaminants, such as bacteria and cellular debris. The composition of claim 1 thus contains a much lower amount of bacterial contaminants (both viable and non-viable) than would be present were the pore size small enough to retain bacteria as well as oocysts.

In contrast, while treatment according to Conkle, et al. may be effective for killing bacteria (e.g., Conkle, et al. teach the treatment of their compositions with antibacterial agents such as hydrogen peroxide or sodium hypochlorite), Conkle, et al. fail to teach or suggest removal of non-viable bacteria or bacterial debris (or any remaining live bacteria), whether by tangential flow filtration or otherwise. Nor would the washings described in Conkle, et al. inherently remove non-viable bacteria or bacterial debris. As will be recognized by those skilled in the art, the typical pore size of diafiltration membranes would be small enough to retain most bacteria and bacterial debris.[3] The composition of Conkle, et al. thus can be said to comprise a greater amount of non-viable bacterial contaminants than the composition of claim 1.

Furthermore, there is no recognition anywhere in Conkle, et al. that it would even be desirable to separate oocysts from non-viable bacteria or bacterial debris that may be present in the composition or during processing. In fact, Conkle, et al. fail to even recognize the problems associated with oocyst-containing compositions that comprise non-viable bacteria or

[3] It is known that diafiltration is a technique that uses ultra filtration membranes (*see, e.g.*, Schwartz, "Diafiltration: A Fast, Efficient Method for Desalting, or Buffer Exchange of Biological Samples", Pall Life Sciences, not formerly of record), and the typical pore size of an ultrafiltration membrane is 0.1 to 0.001 µm, as indicated, e.g., by Dhawan, "Ultrafiltration", http://www.appliedmembranes.com/about ultrafiltration.htm (not formerly of record).

bacterial debris, much less how such problems may be addressed.[4] Consequently, in contrast to the composition of claim 1, Conkle, et al. do not disclose oocyst-containing compositions that are, either expressly or inherently, substantially free of bacterial contaminants which are present in a source but have been separated from the oocysts by tangential flow filtration of an aqueous process medium containing the oocysts and the bacterial contaminants (including non-viable bacterial contaminants) using a filter membrane having a pore size such that sporulated oocysts can not enter the pores, but bacteria can pass through the pores.

As stated in MPEP §2131, a claim is anticipated under 35 U.S.C. §102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. For the reasons discussed above, Conkle, et al. fail to describe each and every element of claim 1, and therefore do not anticipate claim 1.

Examiner's Response to Applicants' Position on Novelty

In response to applicants' arguments, the Examiner has stated that "the purification or production of a product by a particular process does not impart novelty or unobviousness to a product when the same product is taught by the prior art," (emphasis in original), and that "[t]his is particularly true when the properties of the product are not changed by the process in an unexpected manner."[5]

[4] Applicants note that the structural difference between the compositions of claim 1 and the compositions of Conkle, et al. (e.g., a lower amount of non-viable bacterial contaminants) also provides the composition of claim 1 with an unexpected advantage over the compositions of Conkle, et al., i.e., a reduced risk that animals administered the composition will experience a pyrogenic reaction.

[5] *See* p. 8, §B of the final Office action. *See also* p. 3 of the final Office action.

But the premise of this argument fails because here the "same product" is not taught by the prior art. It must be respectfully emphasized that the Examiner's entire rejection is predicated on the erroneous premise that claim 1 fails to specify a structural difference from the Conkle, et al. (and Evans, et al.) references.[6] In particular, the Office action states that limitations such as

> said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores

are merely process limitations.[7] This statement is clearly erroneous. Claim 1, and indeed each pending claim, expressly requires that the composition be "substantially free" of a specified class of bacterial contaminants, i.e., the bacterial contaminants that are present in the source. This is not only a structural difference, but a highly important structural difference from the teachings of both Conkle, et al. and Evans, et al.; not a mere "process limitation." Once the difference is appreciated, it is respectfully submitted that the entire basis for the various rejections falls away.

Applicants acknowledge that the extent of the freedom from bacterial contaminants is expressed in product-by-process language. This measure has been adopted because the extent of freedom from bacterial contamination has not been narrowly quantified and thus cannot be expressed in terms of a numerically defined concentration. However, even as expressed in product-by-process language, the extent of freedom from

[6] See p. 7, section A of the final Office action dated June 13, 2006.
[7] See p. 7-8, sections A and B of the final Office action.

bacterial contaminants comprises a further structural limitation; and such further structural limitation must also be considered in evaluating the novelty and patentability of the claims. As stated in the MPEP:

> The structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product.[8]

Applicants submit that a "distinctive structural characteristic" is imparted by specifying the freedom from bacterial contaminants with reference to tangential flow filtration using:

> "a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores"

Thus, both the express substantial exclusion of "bacterial contaminants which are present in said source" and the product-by-process limitations impose a structural limitation on the claim.

For the reasons set forth above, the substantial exclusion of bacterial contaminants derived from the source of oocysts and the product-by-process limitations impose a structural limitation on the claim and distinguish the teachings of Conkle, et al. under 35 U.S.C. §102.

The authorities cited by the Examiner, i.e., *In re Thorpe*,[9] *In re Marosi*,[10] and *In re Brown*,[11] are not to the contrary. They involve rejections of product-by-process claims under §102 where

[8] MPEP §2113 (emphasis added).
[9] 227 USPQ 964 (Fed. Cir. 1985).
[10] 218 USPQ 289 (Fed. Cir. 1983).
[11] 173 USPQ 685 (C.C.P.A. 1972).

the applicant(s) had failed to identify any structural difference from the prior art, backed up by rejections under §103 on the basis that, if there were any structural differences, they were "slight." But none of these authorities supports either of the propositions for which they are cited in the Office action, i.e., none of these cases supports either: (i) the contention that a purified composition lacks novelty over a corresponding unpurified composition; or (ii) the suggestion that "unexpected properties" are required for novelty.

Applicants further note that MPEP §2144.04 states:

> Pure materials are novel *vis-à-vis* less pure or impure materials because there is a difference between pure and impure materials. Therefore the issue is whether claims to a pure material are unobvious over the prior art.

In the final Office action, the Examiner declined to apply MPEP §2144, stating that this section focuses on rationale for supporting a 103(a) rejection, whereas the rejection over Conkle, et al. is set forth under §102, not §103.[12] Applicants respectfully note that, while some provisions of the MPEP relate to §103(a), MPEP §2144.04 supports the proposition that pure materials are novel as compared to less pure or impure materials, because there is a difference between pure and impure materials.

This principle is further supported by *In re Bergstrom*, 427 F.2d 1394, 166 USPQ 256 (CCPA 1970), where the PTO had rejected claims to certain pure prostaglandin compounds for lack of novelty in light of the material from which it was extracted. On appeal, the court held:

> We need not decide the merits of that matter, for the fundamental error in the Board's position, as we see it, is the analysis and answer it gave to the sole issue it accurately posed -- 'whether the *claimed* pure materials are *novel* as compared to the *less pure* materials of the reference'...It seems to us that the answer to that question is self-evident: by definition, pure materials necessarily differ from *less* pure or impure materials...[13]

Applicants thus submit that claim 1 is patentable under 35 U.S.C. §102 over Conkle, et al.

In the Response to Arguments section, the final action also states that "the well accepted meaning for the term 'purify' or 'purified' means 'to clear from material defilement or imperfection or to rid unwanted contaminants.' Therefore, the prior art does teach the removal of unwanted contaminants."[14] By this statement, the Examiner appears to be suggesting that the compositions of Conkle, et al. are "purified," and therefore anticipate claim 1. But the contention fails because the unwanted contaminants removed by Conkle, et al. is the oxidant, not the bacterial contaminants that are excluded by claim 1.

As noted above, Conkle, et al. describe washing the oocysts to reduce the residual oxidant concentration to an acceptable level. While the oxidant (e.g., sodium hypochlorite) may be an "unwanted contaminant" according to the Examiner's definition of purify, the removal of oxidant from the oocyst compositions of Conkle, et al. does not undermine the patentability of applicants' claim 1. In this regard, it should be remembered that claim 1 does not state that the composition is "purified," but rather, requires the composition be "substantially free of

---

[12] See p. 10, §C of the final Office action.
[13] *Bergstrom*, 166 USPQ at 262 (emphasis added by court).
[14] *See* p. 9, section C of the final Office action.

bacterial contaminants [which include both viable and non-viable contaminants] which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants…" For the reasons set forth above, Conkle, et al. simply fail to disclose such a composition.

Furthermore, the Examiner appears to be misconstruing applicants' arguments. Applicants have not stated that Conkle, et al. do not remove any unwanted material from the compositions described therein. But applicants have identified a structural difference between the composition set forth in claim 1 and the oocyst-containing compositions of Conkle, et al. In particular, Conkle, et al. fail to disclose oocyst-containing compositions that are "substantially free of bacterial contaminants," including both viable and non-viable contaminants.

Examiner's Comments Relating to Obviousness under §103

The Examiner also makes several arguments that relate to obviousness under §103 rather than novelty under §102, although the Office action contains no express obviousness rejection over Conkle, et al. alone. In this category is the contention that a showing of "unexpected properties" or some other secondary consideration is necessary to establish novelty.[15] Contrary to the Examiner's assertion, novelty requires nothing unexpected, only that the claimed subject matter differs from what can be found in the four corners of any single reference.

"Unexpected properties" is an issue which can arise in the context of obviousness. Unexpected properties are not a requirement for non-obviousness; but they can be relevant as secondary evidence overcoming a rejection for *prima facie*

[15] *See* p. 9, §B and p. 3 of the final Office action.

obviousness as made under the three part inquiry mandated by *Graham v. John Deere*, 383 U.S. 1 (1966). *Cf. Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d. 1530 (Fed. Cir. 1983).

In the instant application, the Examiner has neither entered a rejection under §103(a) based on Conkle alone nor offered any basis for *prima facie* obviousness of a coccidiosis vaccine which is substantially free of bacterial contaminants of the type that are universally present in the source from which the oocysts are derived. In the absence of *prima facie* obviousness, there is no burden on applicants to offer secondary evidence of any sort, whether by commercial success, failure of others, unexpected properties, or otherwise.

While section B of the final action requires a showing of unexpected properties or other secondary considerations in the context of the §102 rejection over Conkle, et al.,[16] section D of the action makes seemingly contradictory statements (p. 10). Specifically, the Examiner has agreed with applicants that a rejection under §103 has not been made, and further stated that applicants' remarks relating to unexpected results are irrelevant to a §102 rejection. Thus, the Examiner appears to be acknowledging in Section D that a showing of unexpected properties is not required to overcome a rejection under §102, while requiring in Section B that applicants show unexpected properties to overcome the present §102 rejection.

In another passage that could be relevant only to a rejection for obviousness, the final Office action suggests a need for side by side comparison of the claimed vaccine with Conkle, et al.[17] However, the need for such comparison could arise only if there were *prima facie* obviousness, which has not been shown. Even if *prima facie* obviousness had been shown,

[16] *See* p. 9, section B and p. 3 of the final Office action.

side by side comparison would be needed only if experimental evidence were necessary to establish an unobvious difference from the prior art. Here the substantial absence of bacterial contamination is a material and unobvious difference, and flowing from that difference is an important difference in potential pyrogenicity.

Claim 1 is thus patentable over Conkle, et al. under §102.

Claims 4-8, 14-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 depend either directly or indirectly from claim 1 and are thus patentable for the same reasons as set forth above for claim 1 as well as for the additional elements they require.

Claim 9 is similar to claim 1, except the composition comprises at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate. Claim 10 is similar to claim 1, except the composition comprises less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst and has no limitation on the amount of oocysts per milliliter. Claims 9 and 10, as well as claims 11-13 which depend either directly or indirectly from claim 10, are thus patentable for the same reasons as set forth above for claim 1, as well as for the additional elements they require.

Claims 30 and 142

Claims 30[18] and 142[19] are indirectly dependent on claim 1, and are thus patentable for the same reasons as set forth above for claim 1. Furthermore, the Examiner's evaluation of these

[17] *See* p. 11 of final Office action.
[18] Claim 30 is as follows: "A composition as set forth in claim 29, further comprising a composition that ameliorates a decline in post challenge performance."

claims appears to be prejudiced by misinterpretation thereof. The final Office action states that "[c]laim limitations such as 'the composition ameliorates a decline or decrease in post challenge performance' ... are being viewed as inherent and as a limitation of intended use."[20]

Claims 30 (dependent on claim 29) and 142 (dependent on claim 137) are directed to compositions which further comprise, as a component thereof, a composition which ameliorates a decline or decrease in post challenge performance (i.e. an ameliorating composition). Examples of compositions which ameliorate a decline or decrease in post-challenge performance include, for example, cytokines, growth factors, chemokines, mitogens, and adjuvants. Specific examples of ameliorating compositions are set forth on page 44 of the Specification.

The phrase "which ameliorates a decline [or decrease] in post challenge performance" thus does not specify a mere property of the composition as a whole, but instead defines an additional component of that composition by a functional characteristic which that component possesses. Such "ameliorating composition" is a component that is included in the sporulated oocyst containing compositions of claims 29 and 137 to provide the compositions claimed in claims 30 and 142. The phrase "which ameliorates a decrease [or decline] in post challenge performance" thus does not refer to a mere intended use, but rather, to an ameliorating composition which is a component of the compositions of claims 30 and 142.

In addition, the Examiner has provided no evidence to support the contention that the claim limitation "the

---

[19] Claim 142 is as follows: "A composition as set forth in claim 137 comprising a composition which ameliorates a decrease in post challenge performance."
[20] *See* p. 2 of final Office action.

composition ameliorates a decline or decrease in post challenge performance" is inherent in Conkle, et al. This contention apparently flows from reading "composition" in this context as a property of the vaccine as a whole rather than as a component thereof as explained above. In any case, a finding of inherency cannot be based on mere assumptions by the Examiner. Rather, to establish inherency, "the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art."[21] The final Office action provides no such basis.

In contrast to the compositions of claims 30 and 142, an ameliorating composition is not present as a component of the sporulated oocyst suspensions of Conkle, et al. Rather, the oocysts described in Conkle, et al. are merely suspended in an oxidant during sporulation, and then may be washed using water following sporulation. The compositions of Conkle, et al. cannot be said to necessarily comprise a composition that ameliorates a decrease or decline in post challenge performance. Claims 30 and 142 are thus novel and patentable for this further reason.

Claim 113

Claim 113, directed to a kit comprising the composition of claim 1 and instructions for administration of the composition to an animal, depends from claim 1. Claim 113 is thus patentable for the same reasons as set forth above for claim 1 as well as for the additional elements it requires.

In the final Office action, the Examiner has supported the rejection of claim 113 by stating that a package insert, such as

[21] MPEP §2112.

instructions, does not lend patentable weight to the claim, absent a functional relationship between the instructions and the composition. The action further states that the instructions are a limitation of intended use, that the composition of Conkle, et al. is capable of performing the intended use, and that it therefore meets the claim.[22]

Applicants submit that the instructions in the kit of claim 113 do not constitute a mere intended use, but instead are functionally related to the composition, and therefore should be given patentable weight. As stated by the Federal Circuit in *In re Gulack*, "Under section 103, the board cannot dissect a claim, excise the printed matter from it, and declare the remaining portion of the mutilated claim to be unpatentable. The claim must be read as a whole."[23] Furthermore, "[t]he fact that printed matter by itself is not patentable subject matter, because non-statutory, is no reason for ignoring it when the claim is directed to a combination."[24]

The compositions of the invention may be administered by a variety of routes, and may require dilution before administration.[25] The instructions in claim 113 deal with these physical alternatives and, thus, allow the user of the kit to gain the additional benefit of a properly prepared and administered composition. Claim 113 is thus patentable under 35 U.S.C. §102 over Conkle, et al. for this additional reason.

---

[22] *See* p. 2-3, and p. 10, §E of final Office action.
[23] *In re Gulack*, 217 USPQ 401, 403 (Fed. Cir. 1983).
[24] *In re Miller*, 164 USPQ 46, 49 (C.C.P.A. 1969).
[25] "The vaccine may be concentrated, requiring dilution before administration, or the vaccine may be ready for administration. The concentrated embodiment of the instant invention may be diluted with any suitable diluent to concentrations suitable for various forms of administration, including intra-yolk sac administration, per os, oral gavage, delivery via spray cabinet, or top-fed via spray onto food, such as OASIS Hatchling Supplement." Specification, p. 46, ln. 15-20.

Claims 114-116 and 118-119 depend directly or indirectly from claim 113 and are thus patentable for the same reasons as set forth above for claim 113 as well as for the additional elements they require.

Claim 139

The final Office action rejects claim 139[26] on the basis that the phrase "a ratio defined by the minimum immunizing dose and amount determined by storage [half]-life determinations" is inherent and is a limitation of intended use.[27]

As discussed above, claim 139 depends indirectly on claim 1 and is thus patentable for the same reasons as set forth above for claim 1. It is respectfully submitted that the patentability of claim 139 is fully established on the same basis as claim 1, so that no further response is properly necessary.

Additionally, applicants respectfully submit that the phrase "...a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is a quantification of a dosage amount contained in the composition, not a mere limitation of intended use. Such quantification cannot be found inherently in Conkle, et al. based on the reference's general disclosure that encysted protozoa oocysts including *Eimeria maxima*, *E. mitis*, *E. tenella*, *E. acervulina*, *E. brunetti*, *E. necatrix*, *E. praecox*, and mixtures thereof can be given in a single vaccine. There is no remote connection between this disclosure and the combination of ratio and amounts that is claimed.

---

[26] Claim 139 is as follows: "A composition as set forth in claim 137 wherein said composition comprises viable sporulated oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* in a ratio defined by the minimum immunizing dose and amount determined by storage half life determinations.

[27] *See* p. 2, and p. 11, §F of final Office action.

Not only is quantification defined by the ratio and amounts specified in claim 139 entirely structural, but it also imparts a critically desirable feature to the claimed composition. Since a certain number of sporulated oocysts cease to be functional as they age, providing a quantity of sporulated oocysts as defined by claim 139 helps to assure that quantity of viable oocysts will be sufficient for the vaccine to be effective when used. For this purpose, the minimum number of sporulated oocysts of each *Eimeria* species in the composition may be determined using the minimum immunizing dose and the storage half-life of the sporulated oocysts. As those skilled in the art will readily understand from applicants' specification, the half life defines the slope of the logarithmic decay curve. Back projection on this curve over a period corresponding to storage life defines the amount of oocysts that must be contained in the original dose package in order to assure that minimum immunizing dose remains on the day of administration.[28] By further supplying the plural oocysts in ratios determined by their respective minimum immunizing doses, the claimed combination avoids supplying an excess of one species while supplying a sufficiency of all three, thus assuring efficacy without compromising bird performance.

The Examiner has again provided no evidence whatsoever to support the contention that the claim limitation "a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is inherent in Conkle, et al. To establish inherency, "the examiner must provide a basis in

[28] "The number of sporulated oocysts per dose is further determined by the estimated half-life of the sporulated oocysts in the storage composition claimed herein. As the sporulated oocysts age a certain number cease to be functional...Therefore, a minimum amount of a single species or combination of sporulated oocysts is added to the compositions for consumption that will

fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art."[29] "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic."[30] The Examiner has provided no such basis. In response to applicants arguments, the final Office action merely makes the general statement: "Vaccines are known as pharmaceutical compositions that are used to immunize subjects and are thereby given in immunizing doses and can include determination by storage half-life determinations."[31] With respect to oocyst half-life, this statement is supported solely by hindsight, not by any reference of record.

Conkle, et al. do not so much as mention the problem of aging of sporulated oocysts during shipping and storage, much less how to determine a suitable amount of oocysts by storage half-life determinations, or ratios based on varying minimum-immunizing doses. Conkle, et al. can thus not be said to describe all the limitations of claim 139, and claim 139 is patentable for this further reason.

In light of the foregoing, applicants respectfully request withdrawal of the rejection of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 under 35 U.S.C. §102, and allowance of these claims.

---

result in the minimum immunizing dose computed as a function of half-life determinations." Id. at ln. 21-27.

[29] MPEP § 2112 (citing *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original)).

[30] MPEP §2112 (citing *In re Rijckaert*, 9 F.3d 1531, 1534 (Fed. Cir. 1993)). MPEP §2112 also states "[i]nherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." (quoting *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999).

[31] *See* p. 11, section F of final Office action.

B. Claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 are patentable under 35 U.S.C. §103(a) over Conkle, et al. (WO 00/50072) in view of Brown, et al. (U.S. Patent No. 6,019,985)

Claim 1 and Conkle, et al. are discussed above.

Brown, et al. is directed to methods for improving immunization against coccidiosis and other bacterial, viral, or parasitic diseases in poultry. The method involves administering a solution of *Propionibacterium acnes* suspended in normal saline to a chick at age day 1 following hatching or, alternatively, injecting *P. acnes* in ovo at about day 18 of development. An anticoccidial vaccine may optionally also be administered to the chick. Significantly, Brown, et al. fail to disclose or suggest an oocyst-containing composition that is

> "substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores."

Rather, Brown, et al. is apparently relied on by the Examiner solely for its disclosure of *P. acnes*. Brown, et al. adds nothing to the teachings of Conkle, et al. Nor has the Examiner identified any other teaching of Brown, et al. that is relevant to the compositions as claimed herein. Thus, citation of the Brown, et al. reference would appear to have relevance only with respect to claims 23-28, 30, 142, and 143, which call for a component composition which ameliorates a decline in post-challenge performance, and specifically to claims 26-28 and 143 which expressly call for the presence of *P. acnes*.

In any event, applicants respectfully submit that all claims are patentable over Conkle et al., and over any combination of Conkle, et al. and Brown, et al. under §103.

As explained above, the express exclusion of "bacterial contaminants which are present in said source" and the product-by-process limitations in claim 1 impose structural limitations on the claim that distinguish it from the cited references. In particular, the composition of claim 1 comprises a lower amount of non-viable bacterial contaminants than the composition of Conkle, et al. alone, or in combination with the *P. acnes* described in Brown, et al. Since there is no disclosure or suggestion in either Conkle, et al. or in Brown, et al. of oocyst containing compositions that are substantially free of bacterial contaminants which are present in a source but have been separated from the oocysts by tangential flow filtration of an aqueous process medium containing the oocysts and the bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but the bacterial contaminants can pass through the pores, the cited references fail to teach or suggest all the limitations of claim 1.

Furthermore, there is no statement or suggestion in either of the cited references of the desirability of separating oocysts from non-viable bacterial or other contaminants that may be present in the composition or during processing. As discussed above, Conkle, et al. are satisfied with killing the bacteria and do nothing to remove non-viable bacterial contaminants. They merely disclose washing oocysts following sporulation to reduce the residual oxidant concentration to an acceptable level. There is no recognition in Conkle, et al. of the desirability of producing a composition with a reduced

amount of non-viable bacterial contaminants nor any suggestion as to how such a composition could be produced. There is likewise no such recognition in Brown, et al., which merely discloses administering *P. acnes* to chicks in ovo or following hatching. Brown, et al. state that hatched chicks may also be administered an anti-coccidial vaccine in combination with the *P. acnes*, but do not disclose anything about dosage, or for that matter anything about the composition of the vaccine beyond the fact that it contains "killed or weakened pathogenic microorganisms."[32] Brown, et al. do not suggest removing any non-viable bacterial contaminants from the vaccine, much less reducing them to the level that is achieved by tangential flow filtration as defined in applicants' claims.

In addition, the composition of claim 1 provides an advantage over other compositions (such as the composition of Conkle, et al. alone or in combination with the *P. acnes* of Brown, et al.) in that the lower amount of non-viable bacterial contaminants reduces the risk that animals administered the composition will experience a pyrogenic reaction. Applicants thus submit that the composition of claim 1 has an unexpected and unique property (in this instance lower amount of non-viable bacterial contaminants that results in freedom from an adverse side effect inherent in the compositions of the cited references) that further distinguishes it from the compositions disclosed in the cited references.

Conkle, et al. (alone or in combination with the *P. acnes* of Brown, et al.) create no basis for one skilled in the art to expect that a coccidiosis vaccine comprising oocysts but substantially free of bacterial contaminants might be provided, or could feasibly be provided, or would serve any purpose if it

[32] Brown, et al. at col. 2, lines 46-47.

were provided. Only with the hindsight afforded by applicants' invention can it be seen that there is any need or purpose to provide a vaccine free of bacterial contaminants that include dead bacteria and cellular debris. And only with the hindsight afforded by applicants teachings can it be seen that such a vaccine can feasibly be provided, that it can be produced by tangential flow filtration using a membrane of a certain pore size, and that it provides the important advantage of being less susceptible to creating pyrogenic reactions than the vaccine of Conkle, et al. Thus, all claims should be deemed patentable under the authority of *In re Wakefield*, 422 F.2d 897, 164 USPQ 636 (CCPA 1970), in which a claim reciting "synthetic rubber" was held patentable over a disclosure of otherwise identical subject matter which comprised "natural rubber," despite the absence of any identified structural difference. As stated by Judge Lane:

> We now turn to the examiner's view adopted by the Board, that the synthetic product is so similar to the natural product, purified to the extent allegedly shown in Davis, as to be '*prima facie* obvious.' We would agree with this conclusion as a tentative one based on similarity of structure and gross characteristics. However, such tentative conclusions of obviousness are rebutted in those instances where there was, at the time the invention was made, no known method or obvious method of making the claimed composition, or where the claimed composition is found to possess unexpected characteristics.[33]

A fortiori, the vaccine of claim 1 herein should be found patentable where it recites definitive structural characteristics which distinguish it from Conkle, et al. and Brown, et al., where Conkle, et al. and Brown, et al. fail to suggest any method for producing the vaccine as defined in the

[33] *Wakefield*, 422 F.2d at 903.

claim, where reducing bacterial contamination provides a benefit of reducing the potential pyrogenicity, and where Conkle, et al. and Brown, et al. fail even to recognize the problem that can be caused by dead organisms and/or cellular debris or any need to deal with it.

In light of the foregoing, applicants respectfully submit that claim 1 is patentable over Conkle, et al. and Brown, et al., either alone or in combination.

Claims 4-8, 14-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 depend either directly or indirectly from claim 1 and are thus patentable for the same reasons as set forth above for claim 1 as well as for the additional elements they require.

Claims 9 is similar to claim 1, except the composition contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate. Claim 10 is similar to claim 1, except the composition contains less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst and has no limitation on the amount of oocysts per milliliter. Claims 9 and 10, as well as claims 11-13 which depend either directly or indirectly from claim 10, are thus patentable for the same reasons as set forth above for claim 1, as well as for the additional elements they require.

Claims 23, 30, and 142

Claims 23, 30, and 142 are indirectly dependent on claim 1, and are thus patentable for the same reasons as set forth above for claim 1. Furthermore, applicants again note that the Examiner has appeared to misinterpret claims 23, 30, and 142, stating that claim limitations such as "the composition ameliorates a decline in post-challenge performance" is being viewed as a limitation of intended use.

As noted above, the phrase "which ameliorates a decrease [or decline] in post-challenge performance" does not specify a mere property of the composition as a whole, but instead defines an additional component of that composition by a functional characteristic which that component possesses. The phrase "which ameliorates a decrease [or decline] in post-challenge performance" thus does not refer to a mere intended use, but rather, to an ameliorating composition which is a component of the composition of claims 23, 30, and 142.

Claim 139

The Examiner has also stated with regard to claim 139 that the phrase "a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is a limitation of intended use.[34]

Claim 139 depends indirectly from claim 1 and is thus patentable for the same reasons as set forth above for claim 1. Furthermore, for the reasons set forth above, it is respectfully submitted that the phrase "…a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is more than a mere limitation of intended use, but rather is a further structural limitation that quantifies the amounts of *E. acervulina*, *E. maxima*, and *E. tenella* sporulated oocysts and ratios thereof that are present in the claimed composition.

Additionally, the cited references fail to teach or suggest any ratio of *E. acervulina*, *E. maxima*, and *E. tenella*, present in their composition, nor do either of the cited references recognize the problem of aging of sporulated oocysts during shipping and storage, much less how to determine a suitable

[34] *See* p. 12 of the final Office action.

amount of oocysts by storage half-life determinations. Claim 139 is thus patentable over the cited references for this additional reason.

C. Claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 are patentable under 35 U.S.C. §102(b) over Evans, et al. (WO 96/40234)

The substance of claim 1 is discussed above.

Evans, et al. is directed to a method of vaccinating a domesticated bird against coccidiosis comprising administering *in ovo* an effective immunizing dose of live *Eimeria* sporozoites or merozoites, or a mixture thereof. In describing the preparation of the vaccine, Evans, et al. state that oocysts may be suspended in potassium dichromate solution (2.5% w/v) during sporulation, and that the potassium dichromate is removed from the oocysts suspension "by repeated washings of the oocysts, which involves collection of oocysts by centrifugation and resuspending in deionized or distilled water."[35]

In particular, Evans, et al. fail to disclose or suggest a sporulated oocyst-containing composition that is substantially free of bacterial contaminants that are present in a source but that have been separated from the oocysts by tangential flow fitration of an aqueous process medium containing the oocysts and the bacterial contaminants using a filter membrane with a pore size small enough to prevent sporulated oocysts from entering the pores, but large enough to allow bacteria to pass through the pores.

For example, applicants note that Evans, et al. do not even disclose the use of tangential flow filtration, much less the use of a filter pore size small enough to prevent sporulated

[35] *See* Evans, et al. at p. 6, lines 1-5.

oocysts from entering the pores, but large enough to allow bacteria to pass through the pores. Evans, et al. do state that repeated washings, which involve collection of oocysts by centrifugation and resuspending in deionized or distilled water, may be used to remove the potassium dichromate from the oocyst suspension,[36] and that repeated washings may be used to remove sodium hypochlorite from the oocysts.[37] But Evans, et al. do not suggest that such washings would remove non-viable contaminants from the compositions, and there is no basis in the record to infer that the process described by Evans, et al. might inherently remove non-viable bacterial contaminants. In this regard, applicants note that the centrifugation of an oocyst suspension followed by resuspending in water will not necessarily remove non-viable bacterial contaminants to the extent the tangential flow filtration described in claim 1 does; and, in any case, Evans, et al. fail to disclose centrifugation conditions that would be effective to retain bacterial contaminants in the centrate.

Furthermore, Evans, et al. do not suggest or recognize the desirability of separating the oocysts from non-viable bacterial or other contaminants that may be present in the oocyst suspension. Evans, et al. fail to even recognize the problems associated with oocyst-containing compositions that comprise non-viable bacteria or bacterial debris, much less how such problems may be addressed.

Evans, et al. thus do not teach or suggest compositions that comprise sporulated oocysts that are substantially free of bacterial contaminants which are present in a source but have been separated from the oocysts by tangential flow filtration of an aqueous process medium containing the oocysts and the

[36] *See* Evans, et al. at p. 6, lines 1-4.

bacterial contaminants (including non-viable bacterial contaminants) using a filter membrane having a pore size such that sporulated oocysts can not enter the pores, but bacteria can pass through the pores. Nor would the washings described by Evans, et al. inherently remove bacterial contaminants (including non-viable contaminants) from the sporulated oocysts. Evans betrays no consciousness of a need or purpose for removing bacterial contaminants and does not begin to specify conditions that might be effective for their removal.

Taking a contrary view, applicants note that in the Response to Arguments section of the final Office action, the Examiner has asserted that Evans, et al. teach the removal of contaminants from the suspension comprising oocysts, citing p. 7 of Evans, et al.[38] Applicants respectfully note that p. 7 of Evans, et al. is describing the preparation and purification of merozoites, not oocysts, from host cellular debris. Thus, the Examiner's assertion is in error. The passage cited is clearly not a disclosure of the removal of contaminants from a sporulated oocyst containing suspension.

As stated in MPEP §2131, a claim is anticipated under 35 U.S.C. §102 only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. For the reasons discussed above, Evans, et al. fail to describe each and every element of claim 1, and therefore do not anticipate claim 1.

Examiner's Response to Applicants' Position on Novelty

Additionally, the Examiner has again stated that "the purification or production of a product by a particular process does not impart novelty or unobviousness to a product when the

[37] *See* Evans, et al., p. 6, lines 6-7.

same product is taught by the prior art," and has required applicants to show an unexpected property of the claimed product, such as freedom from some restrictive element or adverse side effects inherent in the product of the prior art.[39] But, as explained above, the premise of this argument fails because here the "same product" is not taught by Evans, et al. For the reasons set forth above, applicants again submit that both the express exclusion of "bacterial contaminants which are present in said source" and the product-by-process limitations in claim 1 impose structural limitations on the claim that distinguish it from the cited reference. In particular, the oocysts in the composition of claim 1 contain a much lower amount of both viable and non-viable bacterial contaminants than would be present were the pore size used during tangential flow filtration small enough to retain bacteria as well as oocysts.

Furthermore, contrary to the Examiner's assertion, applicants are not required to show any unexpected property of the claimed composition to overcome the instant novelty rejection. As noted above, a showing of "unexpected properties" is not necessary to establish novelty, but, rather, can be used as secondary evidence to overcome a rejection for *prima facie* obviousness. Since the Examiner has neither entered a rejection under §103(a) based on Evans, et al. alone nor offered any basis for prima facie obviousness of a coccidiosis vaccine comprising sporulated oocysts which is substantially free of bacterial contaminants, there is no burden on applicants to offer secondary evidence.

The final Office action also suggests the need for side-by-side comparison of the claimed vaccine with Evans, et al.[40]

---

[38] *See* p. 21, §B of the final Office action.
[39] *See* p. 17, and p. 20-21, §B of the final Office action.
[40] *See* p. 21 of the final Office action.

However, as noted above, the need for such comparison could arise only if there were *prima facie* obviousness, which has not been shown (or for that matter even asserted), and then only if experimental evidence were necessary to establish an unobvious difference from the prior art. As discussed above, in the present case, the substantial absence of bacterial contamination in the composition of claim 1 is a material and unobvious difference from the compositions of Evans, et al., and flowing from that difference is an important difference in potential pyrogenicity.

Since Evans, et al. fail to describe each and every element of claim 1, Evans, et al. do not anticipate claim 1. Applicants thus submit that claim 1 is patentable under 35 U.S.C. §102(b) over Evans, et al.

Claims 4-8, 14-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 depend either directly or indirectly from claim 1 and are thus patentable for the same reasons as set forth above for claim 1 as well as for the additional elements they require.

Claim 9 is similar to claim 1, except the composition contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate. Claim 10 is similar to claim 1, except the composition contains less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst and has no limitation on the amount of oocysts per milliliter. Claims 9 and 10, as well as claims 11-13 which depend either directly or indirectly from claim 10, are patentable for the same reasons as set forth above for claim 1.

Claims 30, 113-116, 118-119, 139, and 142

The final Office action also reiterates previous comments that the phrase "the composition ameliorates a decline or decrease in post-challenge performance" (as applied to claims 30 and 142), that the phrase "a ratio is defined by the minimum immunizing dose and amount determined by storage [half]-life determinations" (as applied to claim 139), and that kits and package inserts (as applied to claims 113-116 and 118-119) are being viewed as limitations of intended use.[41] In response to these comments, applicants refer to the arguments made above with respect to Conkle, et al., and submit that a similar line of reasoning applies in the context of the Evans, et al. reference.

In light of the foregoing, applicants respectfully request withdrawal of the rejection of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 under 35 U.S.C. §102(b) and allowance of these claims.

D. Claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 are patentable under 35 U.S.C. §103(a) over Evans, et al. (WO 96/40234) in view of Brown, et al. (U.S. Patent No. 6,019,985)

Claim 1, Evans, et al., and Brown, et al. are discussed above.

Brown, et al. is apparently relied on by the Examiner primarily as suggesting the incorporation of *P. acnes* into the compositions of Evans, et al. However, other than the disclosure of *P. acnes*, the Examiner has not identified any other teaching of Brown, et al. that is relevant to the compositions as claimed herein. Thus, applicants again note

[41] *See* p. 17 of the final Office action.

that citation of the Brown, et al. reference would appear to have relevance only with respect to claims 23-28, 30, 142, and 143, which call for a component composition which ameliorates a decline in post-challenge performance, and specifically to claims 26-28 and 143 which expressly call for the presence of *P. acnes*.

In any event, applicants respectfully submit that all claims are patentable over Evans, et al., and over any combination of Evans, et al. and Brown, et al. under §103.

For the reasons set forth above, applicants again submit that the express exclusion of "bacterial contaminants which are present in said source" and the product-by-process limitations in claim 1 impose a structural limitation on the claim that distinguishes it from the cited references. In particular, the composition of claim 1 contains a much lower amount of bacterial contaminants (both viable and non-viable) than would be present were the pore size small enough to retain bacteria as well as oocysts. Since there is no disclosure or suggestion in either Evans, et al. or in Brown, et al. (nor any motivation to modify the cited references) of oocyst containing compositions that are substantially free of bacterial contaminants which are present in a source but have been separated from the oocysts by tangential flow filtration of an aqueous process medium containing the oocysts and the bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but the bacterial contaminants can pass through the pores, the cited references fail to teach or suggest all the limitations of claim 1.

Furthermore, there is no statement or suggestion in either of the cited references of the desirability of separating oocysts from non-viable bacterial or other contaminants that may

be present in the composition or during processing. As discussed above, Evans, et al. disclose repeated washings, which involve collection of oocysts by centrifugation and resuspending in deionized or distilled water, to remove the potassium dichromate from the oocyst suspension or to remove sodium hypochlorite from the oocysts. There is no recognition in Evans, et al. of the desirability of producing a composition comprising sporulated oocysts that has a reduced amount of non-viable bacterial contaminants nor any suggestion as to how such a composition could be produced. There is likewise no such recognition in Brown, et al., which merely discloses administering *P. acnes* to chicks in ovo or following hatching. Brown, et al. state that hatched chicks may also be administered an anti-coccidial vaccine in combination with the *P. acnes*, but do not disclose or suggest removing non-viable bacterial contaminants from the vaccine, much less how a composition with a reduced amount of non-viable bacterial contaminants could be produced.

In addition, as discussed above, the composition of claim 1 provides an advantage over other compositions (such as any sporulated oocyst containing composition of Evans, et al. alone or in combination with the *P. acnes* of Brown, et al.) in that the lower amount of non-viable bacterial contaminants reduces the risk that animals administered the composition will experience a pyrogenic reaction. Applicants thus submit that the composition of claim 1 has an unexpected and unique property (in this instance lower amounts of non-viable bacterial contaminants that results in freedom from an adverse side effect inherent in the sporulated oocyst-containing compositions of the cited references) that further distinguishes it from the compositions disclosed in the cited references.

In light of the foregoing, applicants respectfully submit that claim 1 is patentable over Evans, et al. and Brown, et al., either alone or in combination.

Claims 4-8, 14-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 depend either directly or indirectly from claim 1 and are thus patentable for the same reasons as set forth above for claim 1 as well as for the additional elements they require.

Claims 9 is similar to claim 1, except the composition contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate. Claim 10 is similar to claim 1, except the composition contains less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst and has no limitation on the amount of oocysts per milliliter. Claims 9 and 10, as well as claims 11-13 which depend either directly or indirectly from claim 10, are thus patentable for the same reasons as set forth above for claim 1.

Claims 23, 30, and 142

Claims 23, 30, and 142 are indirectly dependent on claim 1, and are thus patentable for the same reasons as set forth above for claim 1. Furthermore, applicants again note that the Examiner has appeared to misinterpret claims 23, 30, and 142, stating that claim limitations such as "the composition ameliorates a decline in post-challenge performance" is being viewed as a limitation of intended use.[42] For the reasons set forth above, applicants respectfully submit that the phrase "which ameliorates a decrease [or decline] in post-challenge performance" does not refer to a mere intended use, but rather, to an ameliorating composition which is a component of the composition of claims 23, 30, and 142.

[42] *See* p. 22 of the final Office action.

Claim 139

The Examiner has also again stated with regard to claim 139 that the phrase "a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is a limitation of intended use.[43]

As discussed above, claim 139 depends indirectly from claim 1 and is thus patentable for the same reasons as set forth above for claim 1. Furthermore, for the reasons set forth above, it is respectfully submitted that the phrase "…a ratio defined by the minimum immunizing dose and amount determined by storage half-life determinations" is more than a mere limitation of intended use, but rather is a structural limitation that quantifies the amounts of *E. acervulina, E. maxima,* and *E. tenella* sporulated oocysts and ratios thereof that are present in the claimed composition.

Additionally, the cited references fail to teach or suggest any ratio of *E. acervulina, E. maxima,* and *E. tenella*, present in their compositions, nor do either of the cited references recognize the problem of aging of sporulated oocysts during shipping and storage, much less how to determine a suitable amount of oocysts by storage half-life determinations. Claim 139 is thus patentable over the cited references for this additional reason.

[43] *See* p. 22 of the final Office action.

VIII. Conclusion

Anticipation of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154 has not been established pursuant to 35 U.S.C. § 102, because the Examiner has failed to show a prior art reference disclosing each and every element of claims 1, 4-22, 29-30, 113-116, 118-119, 136-142, 146, 148-150, and 153-154. Furthermore, a prima facie case of obviousness has not been established pursuant to 35 U.S.C. § 103, because the cited art fails to disclose, teach and/or suggest all the elements of claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154. For these reasons, and for those more fully stated above, applicants respectfully request the rejections be reversed and claims 1, 4-30, 113-116, 118-119, 136-143, 146, 148-150, and 153-154 be allowed.

The Commissioner is hereby authorized to charge $500 for the appeal brief and any additional fees which may be required to Deposit Account No. 19-1345.

Respectfully submitted,

/John K. Roedel, Jr./

John K. Roedel, Jr., Reg. No. 25,914
SENNIGER, POWERS
One Metropolitan Square, 16th Floor
St. Louis, Missouri 63102
(314) 231-5400

JKR/LJH/cms
By EFS

CLAIMS APPENDIX

1. (previously presented) A composition for the prevention or control of coccidiosis comprising viable sporulated oocysts that are derived from an oocysts source comprising bacterial contamination and comprise at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains at least about 10,000 oocysts per milliliter and less than about 0.4% by weight of alkali metal dichromate, said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores.

2. (cancelled)

3. (cancelled)

4. (previously presented) A composition as set forth in claim 1 wherein the composition contains less than about 0.2% by weight of alkali metal dichromate.

5. (original) A composition as set forth in claim 4 wherein the composition contains less than about 0.1% by weight of alkali metal dichromate.

6. (previously presented) A composition as set forth in claim 1 wherein said composition is substantially free of alkali metal dichromate.

7. (original) A composition as set forth in claim 1 wherein said composition contains less than about 0.3% by weight of dichromate ion.

8. (original) A composition as set forth in claim 7 wherein said composition contains less than about 0.15% by weight of hexavalent chromium.

9. (previously presented) A composition for the prevention or control of coccidiosis comprising viable sporulated oocysts that are derived from an oocysts source comprising bacterial contamination and comprise at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains at least about 300 oocysts per milliliter and less than about 0.002% by weight of alkali metal dichromate, said composition being substantially free of

bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores.

10. (previously presented) A composition for the prevention or control of coccidiosis comprising viable sporulated oocysts that are derived from an oocysts source comprising bacterial contamination and comprise at least one species of protozoa known to cause coccidiosis, wherein said composition is sterile and contains less than about 5.0×10^{-3} µg of alkali metal dichromate per oocyst, said composition being substantially free of bacterial contaminants which are present in said source but have been separated from said oocysts by tangential flow filtration of an aqueous process medium containing said oocysts and said bacterial contaminants using a filter membrane having a pore size such that sporulated oocysts cannot enter the pores, but said bacterial contaminants can pass through the pores.

11. (original) A composition as set forth in claim 10 wherein said composition is sterile and contains less than about 3.8×10^{-3} µg of alkali metal dichromate per oocyst.

12. (original) composition as set forth in of claim 11 wherein said composition is sterile and contains less than about 1.3×10^{-3} µg of alkali metal dichromate per oocyst.

13. (original) A composition as set forth in of claim 12 wherein said composition is sterile and contains less than about 6.3×10^{-5} µg of alkali metal dichromate per oocyst.

14. (original) A composition as set forth in claim 1, further comprising a diluent.

15. (original) A composition as set forth in claim 14, wherein the diluent comprises water.

16. (original) A composition as set forth in claim 15, wherein the aqueous diluent comprises 0.5X phosphate buffered saline.

17. (original) A composition as set forth in claim 16 further comprising a buffer.

18. (original) A composition as set forth in claim 17, wherein said buffer is selected from the group consisting of phosphate buffer, bicarbonate buffer, citric acid and tris buffers.

19. (previously presented) A composition as set forth in claim 17, wherein said buffer controls pH between about 7.0 and about 7.8.

20. (original) A composition as set forth in claim 14, further comprising a bactericide.

21. (original) A composition as set forth in claim 20, wherein said bactericide is selected from the group consisting of potassium perchlorate, sodium hypochlorite, hydrochlorous acid, sodium hydroxide and antibiotics.

22. (original) A composition as set forth in of claim 21, wherein said composition contains from about 0.1 to about 0.75 wt% potassium perchlorate, and/or from about 0.001 to about 0.01 wt% sodium hypochlorite, and/or from about 1 to about 5 ppm hydrochlorous acid, and/or from about 0.5 to about 1.5 mM sodium

hydroxide and/or from about 20 to about 30 µg/ml gentamicin, in the final composition.

23. (original) A composition as set forth in claim 14, further comprising a composition that ameliorates a decline in post challenge performance.

24. (original) A composition as set forth in claim 23, wherein said composition is selected from the group consisting of Alum, Freund's adjuvant, calcium phosphate, beryllium hydroxide, dimethyl dioctadecyl ammonium bromide, saponins, polyanions, Quil A, inulin, lipopolysaccharide endotoxins, liposomes, lysolecithins, zymosan, propionibacteria, mycobacteria, interleukin 1, interleukin 2, interleukin 4, interleukin 6, interleukin 12, interferon α, interferon γ, and granulocyte colony stimulating factor.

25. (original) A composition as set forth in claim 23, wherein said composition is selected from the group consisting of cytokines, growth factors, chemokines, mitogens and adjuvants.

26. (original) A composition as set forth in claim 25, wherein said composition comprises *Propionibacterium acnes*.

27. (original) A composition as set forth in claim 26, wherein said composition contains at least about 3.0 milligrams (dry weight) of *P. acnes* per milliliter.

28. (original) A composition as set forth in claim 26, wherein said composition contains at least about 30 milligrams (dry weight) of *P. acnes* per milliliter.

29. (previously presented) A composition as set forth in claim 1 comprising:

viable sporulated oocysts of at least one species of protozoa known to cause coccidiosis,

a diluent,

a buffer, and

a bactericide,

wherein said composition contains about 10,000 oocysts pers milliliter and less than about 0.4% weight to volume of alkali metal dichromate.

30. (original) A composition as set forth in claim 29, further comprising a composition that ameliorates a decline in post challenge performance.

Claims 31-112. (cancelled).

113. (previously presented) A kit for the prevention or control of coccidiosis comprising,

the composition of claim 1; and

instructions for administration of said composition to an animal.

114. (original) A kit as set forth in claim 113 containing less than about 0.3% by weight of dichromate ion.

115. (original) A kit as set forth in claim 113 containing less than about 0.15% by weight of hexavalent chromium.

116. (previously presented) A kit as set forth in claim 113 wherein said composition is substantially free of alkali metal dichromate.

117. (cancelled).

118. (previously presented) A kit as set forth in claim 113, further comprising:

a diluent, wherein said diluent is substantially free of alkali metal dichromate; and

instructions for mixing said diluent with said composition to form a mixture.

119. (original) A kit according to claim 118, wherein said diluent comprises a sterile diluent.

Claims 120-135. (cancelled).

136. (previously presented) A composition as set forth in claim 1, said composition having been made by:

introducing into an aqueous sporulation medium oocysts of at least one species of protozoa known to cause coccidiosis;

incubating said oocysts in said aqueous sporulation medium, thereby causing sporulation of oocysts; and

introducing an oxidizing agent into said medium so that the average dissolved oxygen content during sporulation is maintained at at least 30% of saturation;

wherein said composition is substantially free of alkali metal dichromate.

137. (previously presented) A composition as set forth in claim 136 wherein said composition comprises viable sporulated

oocysts from a species of *Eimeria* selected from the group consisting of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*.

138. (previously presented) A composition as set forth in claim 137 wherein said composition comprises viable sporulated oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*.

139. (previously presented) A composition as set forth in claim 137 wherein said composition comprises viable sporulated oocysts of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* in a ratio defined by the minimum immunizing dose and amount determined by storage half life determinations.

140. (previously presented) A composition as set forth in claim 137 wherein said composition comprises at least about 1.25 x 10^{-4} viable sporulated oocysts per milliliter.

141. (previously presented) The composition as set forth in claim 137 wherein said composition is substantially free of added bactericide.

142. (original) A composition as set forth in claim 137 comprising a composition which ameliorates a decrease in post challenge performance.

143. (original) A composition as set forth in claim 142 wherein said composition comprises *Proprionibacterium acnes*.

144. (withdrawn) A composition comprising:

viable, sporulated oocysts of at least one species of coccidial protozoa; and

an anti foaming agent.

145. (withdrawn) A composition as set forth in claim 144 wherein said anti foaming agent is Antifoam A.

146. (previously presented) A composition as set forth in claim 1 wherein said aqueous process medium is an aqueous sporulation medium.

147. (cancelled).

148. (previously presented) A composition as set forth in claim 1 further comprising:

a pharmaceutically acceptable carrier, diluent, or excipient; wherein said composition is substantially free of potassium dichromate.

149. (previously presented) The composition of claim 146 wherein the pore size is approximately 5 microns or greater.

150. (previously presented) The composition of claim 149 wherein the pore size is approximately 10 microns.

151. (cancelled).

152. (cancelled).

153. (previously presented) The composition of claim 1 wherein the pore size is approximately 5 microns or greater.

154. (previously presented) The composition of claim 1 wherein the pore size is approximately 10 microns.

EVIDENCE APPENDIX

Submitted herewith are copies of Schwartz, "Diafiltration: A Fast, Efficient Method for Desalting, or Buffer Exchange of Biological Samples," Pall Life Sciences, and Dhawan, "Ultrafiltration," http://www.appliedmembranes.com/about_ultrafiltration.htm. Neither of these references has previously been made of record.

RELATED PROCEEDINGS APPENDIX

None.

BACK TO BASICS

Contents *(click a link to skip to that section)*

Ultrafiltration

By Dr. Gil Dhawan

Ultrafiltration is a separation process using membranes with pore sizes in the range of 0.1 to 0.001 micron. Typically, ultrafiltration will remove high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecules. Low molecular-weight organics and ions such as sodium, calcium, magnesium chloride, and sulfate are not removed. Because only high-molecular weight species are removed, the osmotic pressure differential across the membrane surface is negligible. Low applied pressures are therefore sufficient to achieve high flux **rates from an ultrafiltration membrane. Flux of a membrane is defined as the amount of** permeate **produced per unit area of membrane surface per unit time. Generally** flux **is expressed as gallons per square foot per day (GFD) or as cubic meters per square meters per day.**

Ultrafiltration membranes can have extremely high fluxes **but in most practical applications the flux varies between 50 and 200 GFD at an operating pressure of about 50 psig in contrast, reverse osmosis membranes only produce between 10 to 30 GFD at 200 to 400 psig.**

Ultrafilter vs. Conventional Filter

Ultrafiltration, like reverse osmosis, is a cross-flow separation process. Here liquid stream to be treated (feed) flows tangentially along the membrane surface, thereby producing two streams. The stream of liquid that comes through the membrane is called permeate. **The type and amount of species left in the** permeate **will depend on the characteristics of the membrane, the operating conditions, and the quality of** feed. **The other liquid stream is called** concentrate **and gets progressively concentrated in those species removed by the membrane. In cross-flow separation, therefore, the membrane itself does not act as a collector of ions, molecules, or colloids but merely as a barrier to these species.**

Conventional filters such as media filters or cartridge filters, on the other hand, only remove suspended solids by trapping these in the pores of the filter-media. These filters therefore act as depositories of suspended solids and have to be cleaned or replaced frequently. Conventional filters are used upstream from the membrane system to remove relatively large suspended solids and to let the membrane do the job of removing fine particles and dissolved solids. In ultrafiltration, for many applications, no prefilters are used and ultrafiltration modules concentrate all of the suspended and emulsified materials.

Concentration Polarization

When a membrane is used for a separation, the concentration of any species being removed is higher near the membrane surface than it is in the bulk of the stream. This condition is known as concentration

polarization and exists in all ultrafiltration and reverse osmosis separations. The result of concentration polarization is the formation of a boundary layer of substantially high concentration of substances being removed by the membrane. The thickness of the layer and its concentration depend on the mass of transfer conditions that exist in the membrane system. Membrane flux and feed flow velocity are both important in controlling the thickness and the concentration in the boundary layer. The boundary layer impedes the flow of water through the membrane and the high concentration of species in the boundary layer produces a permeate of inferior quality in ultrafiltration applications relatively high fluid velocities are maintained along the membrane surface to reduce the concentration polarization effect.

Recovery

Recovery of an ultrafiltration system is defined as the percentage of the feed water that is converted into the permeate, or:

$$R = \frac{P}{F} \times 100$$

Where: R = Recovery
P = Volume of permeate
F = Volume of Feed

Ultrafiltration Membranes

Ultrafiltration Membrane modules come in plate-and-frame, spiral-wound, and tubular configurations. All configurations have been used successfully in different process applications. Each configuration is specially suited for some specific applications and there are many applications where more than one configuration is appropriate. For high purity water, spiral-wound and capillary configurations are generally used. The configuration selected depends on the type and concentration of colloidal material or emulsion. For more concentrated solutions, more open configurations like plate-and-frame and tubular are used. In all configurations the optimum system design must take into consideration the flow velocity, pressure drop, power consumption, membrane fouling and module cost.

Membrane Materials

A variety of materials have been used for commercial ultrafiltration membranes, but polysulfone and cellulose acetate are the most common. Recently thin-film composite ultrafiltration membranes have been marketed. For high purity water applications the membrane module materials must be compatible with chemicals such as hydrogen peroxide used in sanitizing the membranes on a periodic basis.

Molecular-Weight Cutoff

Pore sizes for ultrafiltration membranes range between 0.001 and 0.1 micron. However, it is more customary to categorize membranes by molecular-weight cutoff. For instance, a membrane that removes dissolved solids with molecular weights of 10,000 and higher has a molecular weight cutoff of 10,000. Obviously, different membranes even with the same molecular-weight cutoff, will have different pore size distribution. In other words, different membranes may remove species of different molecular weights to different degrees. Nevertheless, molecular-weight cutoff serves as a useful guide when selecting a membrane for a particular application.

Factors Affecting the Performance of Ultrafiltration

There are several factors that can affect the performance of an ultrafiltration system. A brief discussion of these is given here.

Flow Across the Membrane Surface. The permeate rate increases with the flow velocity of the liquid across the membrane surface. Flow velocity if especially critical for liquids containing emulsions or suspensions. Higher flow also means higher energy consumption and larger pumps. Increasing the flow velocity also reduces the fouling of the membrane surface. Generally, an optimum flow velocity is arrived at by a compromise between the pump horsepower and increase in permeate rate.

Operating Pressure. Permeate rate is directly proportional to the applied pressure across the membrane surface. However, due to increased fouling and compaction, the operating pressures rarely exceed 100 psig and are generally around 50 psig. In some of the capillary-type ultrafiltration membrane modules the operating pressures are even lower due to the physical strength limitation imposed by the membrane module.

Operating Temperature. Permeate rates increase with increasing temperature. However, temperature generally is not a controlled variable. It is important to know the effect of temperature on membrane flux in order to distinguish between a drop in permeate due to a drop in temperature and the effect of other parameters.

Performance of Ultrafiltration Systems

In high purity water systems, ultrafiltration is slowly replacing the traditional 0.2-micron cartridge filters. In Japan, practically all of the semiconductor industry follows this practice. An ultrafiltration membrane with a molecular-weight cutoff of 10,000 has a nominal pore size of 0.003 micron. When an ultrafiltration membrane is used instead of a 0.2-micron cartridge filter, particle removal efficiency is greatly improved. In addition, ultrafiltration membranes are not susceptible to the problem of bacteria growing through them, as is the case with 0.2-micron filters.

In a recent study [1], the performance of an ultrafilter was compared with that of a 0.2-micron cartridge filter. Some of these results are given in Table A.

The Ultrafilter used in the study had a molecular-weight cutoff of 100,000- (pore size 0.006 micron). As the requirements for the quality of high purity water become more stringent, we can expect to see an increasing use of ultrafiltration as a final filter.

Table A

Effectiveness of Ultrafiltration Particle Counts on Waters		
Test Location	0.2 Micron Filtered DI Rinse Water	Unfiltered DI Rinse Water
1	200-300	20-30*
2	175-200	0-25
3	120	5
4	275	125*

*Plumbing after UF not upgraded

Operation and Maintenance

Ultrafiltration system operation and maintenance is similar to that of reverse osmosis systems. Daily records of feed and permeate flow, feed pressure and temperature, and pressure drop across the system should be kept. Membranes should be cleaned when the system permeate rate drops by 10% or more. Feed flow is critical to the operation of ultrafiltration systems. A drop in feed flow may be due to a problem in the prefilter (if any), with the flow control valve, or with the pump itself. When the system is shut down for more than two days, a bacteriocide should be circulated through the membranes. At restart, permeate should be diverted to drain until all the bacteriocide is removed.

Conclusions

Ultrafiltration will find an increasing application in the production of high purity water. The basic principles outlined here should help in the understanding and use of this technology.

Reference

[1]Gaudet, P.W. "Point-of-use Ultrafiltration of Deionized Water and Effects of Microelectronics Device Quality, American Society for Testing and Materials", 1984.

Glossary of Terms

Feed - Liquid to be treated by the ultrafiltration system.

Permeate - Liquid stream that passes through the membrane.

Concentrate - Remaining Portion of the liquid stream after the permeate has been
Recovery - Expressed as percentage, this defines the permeate rate as a fraction of the feed rate. Recovery provides an immediate measure of the maximum concentrations in the system and it affects permeate quality, pump size, power consumption and membrane fouling.
Flux - Permeate flow per unit area of membrane per unit time (gallons/ft²/day)
Rejection - Percent removal of a particular species by the membrane. Expressed as $1-C_P\ C_F$ where C_P is the concentration in t he permeate, and C_F is the concentration in the feed.
Flow Velocity - Rate at which the liquid goes along the membrane surface, expressed in length per unit time (ft/sec).

The Author

Dr. Gil Dhawan is the president of Applied Membranes, Inc. based in Vista, CA. Dr. Dhawan has been involved in the design, development, and marketing of ultrafiltration and reverse osmosis systems for the past 20 years.

For prompt, courteous and reliable service, contact us today!
(760) 727-3711 • Fax (760) 727-4427 • sales@appliedmembranes.com

For comments or suggestions regarding this website, E-mail the Webmaster.

AMI Home Page

Back Up

Membrane Spec Sheets | Membrane Storage | Membrane Preservation
Temp/Pressure Correction | Rejection Chart | Comparison of Membranes
Membrane Warranty | About Ultrafiltration

PALL Life Sciences

Scientific & Technical Report

PN 33289

Diafiltration: A Fast, Efficient Method for Desalting, or Buffer Exchange of Biological Samples

By Larry Schwartz, Senior Technical Manager, Pall Life Sciences

Diafiltration is a technique that uses ultrafiltration membranes to completely remove, replace, or lower the concentration of salts or solvents from solutions containing proteins, peptides, nucleic acids, and other biomolecules. The process selectively utilizes permeable (porous) membrane filters to separate the components of solutions and suspensions based on their molecular size. An ultrafiltration membrane retains molecules that are larger than the pores of the membrane while smaller molecules such as salts, solvents and water, which are 100% permeable, freely pass through the membrane.

This article will cover the concepts of protein concentration and diafiltration. It will compare different ways of performing diafiltration and their impact on process time, volume, product stability, and recovery.

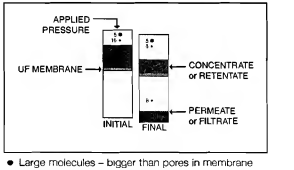
CONCENTRATION

The solution retained by the membrane is known as the concentrate or retentate. The solution that passes through the membrane is known as the filtrate or permeate.

A membrane for concentration is selected based on its rejection characteristics for the sample to be concentrated. As a general rule, the molecular weight cut-off (MWCO) of the membrane should be 1/3rd to 1/6th the molecular weight of the molecule to be retained (3-6X Rule). This is to assure complete retention. The closer the MWCO is to that of the sample, the greater the risk for some small product loss during concentration. The risk increases if diafiltration will also be used since the relative loss depends on the total volume of filtrate that will be generated. Membrane flux rate (filtrate flow rate per unit area of membrane) is related to pore size. The smaller the pores, the lower the flux rate for the same applied pressure. Therefore, when selecting a membrane for concentration / diafiltration, one must consider the time factor versus product recovery. In most biological applications, recovery outweighs the time consideration. The process time can always be reduced by increasing the amount of membrane area used.

Figure 1 below provides an example of concentration. The sample is placed in a device containing a suitable ultrafiltration membrane that will retain the large molecules. Pressure is applied until half the volume has passed through the membrane. The large molecules are retained in half the original volume (concentrate), which also contains half of the salt molecules. The filtrate contains the other half of the salt molecules but none of the large molecules. Therefore, the large molecules are concentrated as liquid and salt are removed. The salt molecule to volume ratio in the concentrate remains constant so the ionic strength of the concentrated solution remains relatively constant.

Figure 1.
2X Concentration of Sample by Ultrafiltration



- Large molecules – bigger than pores in membrane
- Small molecules – salts or solvent

The ionic strength of the concentrate (retentate) solution can subsequently be reduced by "washing" the remaining salt out with water, a process called diafiltration. This is essentially a dilution process and is performed in conjunction with a concentration process. Water is added while filtrate is removed. If the washing solution is another buffer instead of water, the new buffer salt will replace the initial salt in the sample.

For simplicity, the above and subsequent examples use a direct flow filtration device such as a centrifugal concentrator. The same principles apply to cross flow filtration devices such as cassettes and hollow fibers where the retentate is recirculated.

BENEFITS OF DIAFILTRATION

Conventional techniques used for salt removal or buffer exchange such as membrane dialysis and column-based gel filtration can be effective but have limitations. Dialysis procedures can take up to several days, require large volumes of water for equilibration and risk product loss through manual manipulation of the dialysis bags. Gel filtration results in a dilution of the sample and often requires an additional ultrafiltration step to concentrate it back. Adding steps to a process can risk sample loss or possible contamination. With diafiltration, salt or solvent removal as well as buffer exchange can be performed quickly and conveniently. Another big advantage of diafiltration is that the sample is concentrated on the same system, minimizing the risk of sample loss or contamination.

There are several ways to perform diafiltration. While the end result may be the same, the time and volume required to complete the process may vary considerably. It is important to understand the differences in the methods used and when to choose one over the other.

CONTINUOUS DIAFILTRATION

The technique of continuous diafiltration (also referred to as constant volume diafiltration) involves washing out the original buffer salts (or other low molecular weight species) in the retentate (sample) by adding water or a new buffer to the retentate at the same rate as filtrate is being generated. As a result, the retentate volume and product concentration does not change during the diafiltration process. If water is used for diafiltering, the salts will be washed out and the conductivity lowered. If a buffer is used for diafiltering, the new buffer salt concentration will increase at a rate inversely proportional to that of the species being removed.

The amount of salt removed is related to the filtrate volume generated, relative to the retentate volume. The filtrate volume generated is usually referred to in terms of "diafiltration volumes". A single diafiltration volume (DV) is the volume of retentate when diafiltration is started. For continuous diafiltration, liquid is added at the same rate as filtrate is generated. When the volume of filtrate collected equals the starting retentate volume, 1 DV has been processed.

Using continuous diafiltration, greater than 99.5% of a 100% permeable solute can be removed by washing through 6 retentate volumes (6DV) with the buffer of choice.

Molecules that are larger than salts and solvents, but which are still smaller than the pores in the membrane, can also be washed out. The permeability of these molecules, however, may be less than 100%. In such cases, it will take more liquid, i.e. more DV's, to completely wash a partially permeable molecule through the membrane, compared to a 100% permeable molecule. Typically, the larger the molecule, the lower the permeability and the greater the wash volume required.

The permeability of a molecule through a specific membrane can be determined by measuring the concentration of the molecule in the filtrate compared to the concentration in the retentate under specified conditions.

$$\% \text{ permeability} = (\text{Conc.}_{\text{FILTRATE}} / \text{Conc.}_{\text{RETENTATE}}) \times 100$$

Permeability is often described in terms of "Rejection Coefficient" of the membrane, i.e. the membrane's ability to hold back or reject a given molecule from passing through.

$$\text{Rejection Coefficient} = 1 - (\text{Conc.}_{\text{FILTRATE}} / \text{Conc.}_{\text{RETENTATE}}).$$

A rejection coefficient of 1 equals 0% permeability

A rejection coefficient of 0 equals 100% permeability

Permeability will be affected by such factors as transmembrane pressure (TMP), crossflow rate, retentate concentration, pH, and ionic strength, and gel layer formation (concentration polarization). Therefore, the permeability may change during the process.

Table 1 shows the relationship between permeability through a membrane and the number of diafiltration volumes required for removal of permeating species. As noted earlier, a greater volume of buffer is required to remove a molecule that is partially retained. To remove 99.9% of a molecule that is 25% permeable to the membrane requires 9 DV's, while for a 100% permeable species, only 7 DV's are required.

Table 1:
Continuous (Constant Volume) Diafiltration

Diafiltration Volumes	Permeability 100% Rejection Coefficient = 0	Permeability 75% Rejection Coefficient = 0.25
1	63%	53%
2	86%	77%
3	95%	89%
4	98.2%	95%
5	99.3%	97.6%
6	99.7%	98.9%
7	99.9%	99.4%
8		99.7%
9		99.9%

0% - Salts, solvents, buffers, etc.

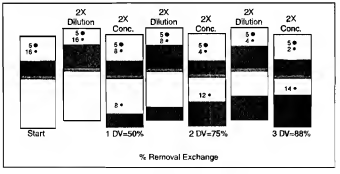
25%- Molecules lower in MW than MWCO of membrane but bigger than salts

DISCONTINUOUS DIAFILTRATION – Sequential Dilution

Discontinuous diafiltration by sequential dilution involves first diluting the sample with water or replacement buffer to a predetermined volume. The diluted sample is then concentrated back to its original volume by ultrafiltration. This process is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent dilution removes more of the small molecules.

As shown in Figure 2, the sample is generally diluted with an equal volume of buffer (1DV). Alternatively, multiple volumes can be added at once, provided the process tank is large enough to hold the entire volume. Diluting the sample usually lowers the viscosity, which may increase the filtrate flux rate.

Figure 2.
Discontinuous Diafiltration – Sequential Dilution

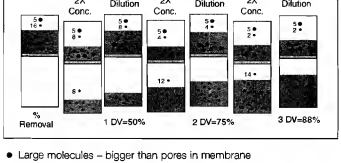


- Large molecules – bigger than pores in membrane
- Small molecules – salts or solvent

DISCONTINUOUS DIAFILTRATION – Volume Reduction

Discontinuous diafiltration by volume reduction reverses this procedure. The sample is first concentrated to a predetermined volume, and then diluted back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent concentration and dilution removes more of the small molecule (Figure 3).

Figure 3.
Discontinuous Diafiltration with Volume Reduction



- Large molecules – bigger than pores in membrane
- Small molecules – salts or solvent

After the last buffer addition to complete diafiltration, the sample may be concentrated before analysis or the next purification step is performed.

The final product, after diafiltration by either method (discontinuous 2X volume reduction or sequential dilution) is at the same volume and concentration as when diafiltration started. The salt concentration has been equally reduced in both examples. However, the volume of diafiltration buffer used by the volume reduction method was half that used in sequential dilution. This is because the initial concentration step reduced the volume in half. A diafiltration volume is equal to the volume where dilution occurs. Therefore, half the volume was required.

This being the case, it would seem that concentrating before diafiltration, by either discontinuous sequential dilution or constant volume diafiltration, should reduce the required diafiltration buffer volume and save time. And in most cases this is true. The factor we have not accounted for is filtrate flux rate, which equates to process time. As the product becomes concentrated, viscosity increases and the filtrate flux rate decreases. The filtrate flux rate varies inversely as the log of the concentration factor.

$$J = k \ln(C_G / C_B)$$

Where

J = Filtrate Flux Rate

k = constant

C_G = gel layer concentration

C_B = retentate (bulk flow) concentration

This becomes very significant as the product concentration (C_B) increases above a few percent and is dependent on the characteristics of the specific molecules that make up the sample. So, although it might take significantly less volume to diafilter a concentrated sample, it could take considerably more time compared to a less concentrated sample. Simple protocols are available to find optimum conditions to maximize productivity.

Table 2.
Salt Reduction from Sample using Volume Reduction or Constant Volume Diafiltration

Diafiltration Volumes	2X Volume Reduction		Continuous Diafiltration (Constant Volume)	
	100% Permeable 0% Retention*	75% Permeable 25% Retention*	100% Permeable 0% Retention*	75% Permeable 25% Retention*
1	50%	41%	63%	53%
2	75%	65.%	86%	77%
3	88%	79%	95%	89%
4	94%	88%	98.2%	95%
5	96.9%	93%	99.3%	97.6%
6	98.4%	95.6%	99.7%	98.9%
7	99.2%	97.4%	99.9%	99.4%
8	99.6%	98.4%		99.7%
9	99.8%	99.0%		99.9%
10	99.9%	99.4%		

*Retention of smaller molecules

0% - Salts, solvents, buffers, etc.

25%- Molecules lower in MW than MWCO of membrane but bigger than salts

CONTINUOUS OR DISCONTINUOUS DIAFILTRATION - WHICH TECHNIQUE SHOULD BE USED?

When deciding which technique to use and where in the process to perform diafiltration, consider the following factors:

1) Initial sample volume, concentration and viscosity

2) Required final sample concentration

3) Stability of sample at various concentrations

4) Volume of buffer required for diafiltration

5) Total processing time

6) Reservoir size available

7) Economics

The choice of which method to use must be based on several criteria. Scale is an important consideration. What we will do at laboratory scale may be very different than at process scale, especially if the process is automated. At lab scale discontinuous diafiltration is often used for simplicity. Continuous diafiltration requires a pump or equipment to add the diafiltration solution at a constant rate. Both techniques can be automated for process applications. If we eliminate the equipment issue and focus on the process. we can compare the differences.

The ionic strength, buffer composition and stabilizer concentration can affect stability of the sample. Diafiltration may remove salts or stabilizing molecules, resulting in protein product denaturation and aggregation. The process of concentrating and diluting a protein solution can also affect molecular interactions resulting in denaturation or aggregation as well as subsequent precipitation and product loss. It is necessary to evaluate the effect of concentration on the product to determine where diafiltration is best performed relative to concentration effects.

Continuous diafiltration offers an advantage over discontinuous diafiltration in that the retentate concentration remains constant. It is often seen as a more gentle process relative to the stability of the product.

WHEN TO PERFORM DIAFILTRATION - BEFORE OR AFTER CONCENTRATION?

We have already seen that concentrating a sample first can significantly reduce the volume of diafiltration solution required. We have also seen that continuous diafiltration takes less volume than discontinuous diafiltration with sequential dilution. Therefore, if the sample is first concentrated to the final concentration required and then continuous diafiltration performed, acceptable results should be obtained.

However, above a certain concentration, filtrate flux rates may become prohibitively slow. It may actually takes longer to diafilter the concentrated sample than it would if the sample were first diluted to reduce the concentration. In this situation, even though continuous diafiltration of the diluted sample requires a greater diafiltration volume, the total processing time would be less due to the faster filtrate flux rate. (Process Time = Filtrate flow rate x Volume)

In general, the optimum retentate concentration for performing (continuous) diafiltration is at:

$\ln (C_G/C_R) = 1$ or $C_{R\ (optimum)} = C_G/e = 0.37C_G$ *

Where

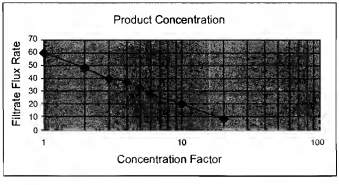
C_G = gel layer concentration

C_R = retentate concentration.

$C_{R(Optimum)}$ = highest retentate concentration where diafiltration should be performed

The C_G value for a sample can be determined from experimentation by concentrating a sample on a membrane and recording and plotting data for filtrate flux rate vs. log concentration (or concentration factor). The curve can then be extrapolated to filtrate flux rate = "0". The C_G value will be the same for this product regardless of the starting concentration or filtrate flux rate.

Figure 4.
Determination of the C_G Value for a product



In this example (Figure 4) the C_G value is a concentration factor of approximately 33X. Therefore the optimal concentration to perform diafiltration would be $0.37C_G$ = 12.2X. If the starting product concentration is 5mg/mL, then diafiltration should be performed when the concentration reaches 61mg/mL. If the final concentration will be less than 61mg/mL, then diafiltration should be performed after concentration, unless it is necessary to remove a specific molecule prior to concentration.

The ultrafiltration product selected may dictate choice of continuous or discontinuous diafiltration. Stirred cells and centrifugal devices are best suited for discontinuous diafiltration because of their mode of operation. Tangential flow devices have the advantage of being useful for either diafiltration technique.

Summary

Diafiltration is a fast and effective technique for desalting or buffer exchange of solutions. It can be performed in a continuous or discontinuous mode. Continuous diafiltration usually takes less volume to achieve the same degree of salt reduction as discontinuous diafiltration with sequential dilution and can be easier to perform.

Continuous diafiltration is also perceived as a kinder and gentler process on active biomolecules. On the other hand, discontinuous diafiltration with volume reduction takes less volume than continuous diafiltration. Concentrating the sample before diafiltration usually reduces the required filtrate volume and saves time. However if the sample viscosity becomes too great, the filtrate flux rate decreases and the process time can increase substantially. Determining the C_G for the sample can help answer the question - At what concentration should I perform diafiltration?

Initial volumes from a few milliliters up to thousasnds of liters can be processed using Pall's Tangential Flow Filtration (TFF) products. Pall's tangential flow systems allow easy addition of membrane surface area, providing the flexibility to reduce processing time or scale up the process. This range of devices and systems provides scalability as well as the flexibility to work with almost any sample volume. Pall's highly selective OMEGA™ polyethersulfone (PES) membranes with narrow pore size distribution and high flow rates provide fast processing time and efficient separation for diafiltration.

* Industrial Ultrafiltration Design and Application of Diafiltration Processes, Beaton & Klinkowski, J. Separ. Proc. Technol., 4(2) 1-10 (1983)

Glossary

Diafiltration: Diafiltration is a technique that uses ultrafiltration membranes to completely remove or lower the concentration of salt or solvent, or to replace buffer salts from solutions containing proteins and other large molecules.

Diafiltration Volume: One diafiltration volume equals the initial volume in which the molecule of interest is suspended. The number of diafiltration volumes required depends on whether the permeating species is freely passing (salts, buffers, solvents) or partially retained.

Continuous Diafiltration: The technique of continuous diafiltration (also referred to as constant volume diafiltration) involves washing out the original buffer salts (or other low molecular weight species) in the retentate (sample) by adding water or a new buffer to the retentate at the same rate as filtrate is being generated

Discontinuous Diafiltration-Sequential Dilution: Discontinuous diafiltration by sequential dilution involves first diluting the sample to a predetermined volume, then concentrating the sample back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent dilution removes more of the small molecules

Discontinuous Diafiltration-Volume Reduction: Discontinuous diafiltration by volume reduction involves first concentrating the sample to a predetermined volume, then diluting the sample back to its original volume with water or replacement buffer. This is repeated until the unwanted salts, solvents, or smaller molecules are removed. Each subsequent concentration and dilution removes more of the small molecule.

PALL Life Sciences

Pall Life Sciences
600 South Wagner Road
Ann Arbor, MI 48103-9019 USA

800.521.1520 toll free in USA
734.665.0651 telephone
734.913.6114 fax

United Kingdom
Europa House
Havant Street
Portsmouth, Hampshire
PO1 3PD

023 92 302600 telephone
023 92 302601 fax

Australia – Lane Cove, NSW
Tel: 02 9428-2333
1800 635-082 (in Australia)
Fax: 02 9428-5610
Austria – Wien
Tel: 043-1-49 192-0
Fax: 0043-1-49 192-400
Canada – Ontario
Tel: 905-542-0330
800-263-5910 (in Canada)
Fax: 905-542-0331
Canada – Québec
Tel: 514-332-7265
800-435-6268 (in Canada)
Fax: 514-332-0996
800-808-6268 (in Canada)
China – P. R., Beijing
Tel: 86-10-8458 4010
Fax: 86-10-8458 4001
France – St. Germain-en-Laye
Tel: 01 30 61 39 92
Fax: 01 30 61 58 01
Lab-FR@pall.com

Germany – Dreieich
Tel: 06103-307 333
Fax: 06103-307 399
Lab-DE@pall.com
India – Mumbai
Tel: 91-22-5956050
Fax: 91-22-5956051
Italy – Milano
Tel: 02-47796-1
Fax: 02-47796-394
or 02-41-22-985
Japan – Tokyo
Tel: 3-3495-8319
Fax: 3-3495-5397
Korea – Seoul
Tel: 2-569-9161
Fax: 2-569-9092
Poland – Warszawa
Tel/Fax: 22-835 83 83
Russia – Moscow
Tel: 095 787-76-14
Fax: 095 787-76-15

Singapore
Tel: (65) 389-6500
Fax: (65) 389-6501
Spain – Madrid
Tel: 91-657-9876
Fax: 91-657-9836
Sweden – Lund
Tel: +46 (0)46 158400
Fax: +46 (0)46 332761
Switzerland – Basel
Tel: 061-638 39 00
Fax: 061-638 39 40
Taiwan – Taipei
Tel: 2-2545-5991
Fax: 2-2545-5990
United Kingdom – Portsmouth
Tel: 023 92 302600
Fax: 023 92 302601
Lab-UK@pall.com

Visit us on the Web at www.pall.com

E-mail us at Lab@pall.com or Lab-UK@pall.com

Filtration. Separation. Solution.℠

2/03, 2.5K, GN02-0629

PN53289